

## THE CARDINI SPECIES GROUP OF THE GENUS DROSOPHILA

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A study of the relationships among species of the *cardini* group of the genus *Drosophila* was undertaken with the aid of a combination of genetic and morphological methods. A purely morphological analysis of species of *Drosophila* is unsatisfactory. In case of morphologically identical species, or sibling species as Mayr (1942) calls them, morphological analysis is clearly impossible. Many such species are known in *Drosophila*, owing chiefly to the work of Patterson and his school (Patterson 1943, and other work). Dobzhansky and Epling (1944) have described *Drosophila persimilis* Dobzhansky and Epling which is morphologically identical with *D. pseudoobscura* Frolova, and Dobzhansky (in press) describes *D. equinoxialis* Dobzhansky which is morphologically very nearly identical with *D. willistoni* Sturtevant.

The following species of the *cardini* group of *Drosophila* have been described: *Drosophila cardini* Sturtevant (1916) from Cuba, *D. cardinoides* Dobzhansky and Pavan (1943) from southern Brazil, *D. polymorpha* Dobzhansky and Pavan (1943) from southern Brazil, and *D. neocardini* sp. n. described below from Amazonas, Brazil.

The chromosome complements of two strains of *D. cardini* Sturtevant from Cuba (not including, however, the type strain) were described by Metz (1923) as consisting of five pairs of rod-like and one pair of dot-like chromosomes. Dobzhansky and Pavan (1943) found in *D. cardinoides* (type strain) two pairs of V-shaped, one pair of rod-shaped, and a pair of dot-like chromosomes; they recognized the morphological similarity of their material and the description of *D. cardini* given by Sturtevant, but proposed the name *cardinoides* because of the dissimilarity of the chromosomes and of the geographic origin. Patterson (1943) and Wharton (1943) found several strains from Southern United States, Mexico, and Guatemala which have the

same chromosome complements as the Brazilian *D. cardinoides* Dobzhansky and Pavan, but classified these strains as *D. cardini* Sturtevant. A strain from Guatemala obtained through the courtesy of Professor J. T. Patterson is morphologically and cytologically similar to, and interbreeds readily with, the Brazilian *D. cardinoides* Dobzhansky and Pavan. The status of the name *D. cardinoides* is, then, in doubt, pending re-examination of the cytology and the breeding behavior of the Cuban representatives of this species group.

The present study is based on the following strains which are maintained in the laboratory: *Drosophila cardinoides* Dobzhansky and Pavan from Iporanga and from Mogi das Cruzes, State of São Paulo, from Teffe, State of Amazonas, from Belem, State of Para, in Brazil, and from Guatemala in Central America. *Drosophila polymorpha* Dobzhansky and Pavan came from Bertoga and from Araras, State of São Paulo, Brazil. *Drosophila neocardini* sp. n., came from Teffe, Amazonas, Brazil.

Although, as shown below, the three species are morphologically distinguishable, the most convincing and conclusive method of classification proved to be their behavior in crosses. After the strains were so classified, it was found that the structure of the male genitalia also furnishes satisfactory criteria for the separation of these three species.

For the initial tests, approximately twenty virgin females of one strain were placed together with twenty freshly hatched males of another strain. If fertile offspring were produced, the strains were considered to belong to the same species. Where no offspring appeared, the flies were transferred to fresh bottles, and after a month had elapsed, the surviving females were dissected and their seminal receptacles were examined under the microscope for the presence of sperm. The data are summarized in Table 1. In Table 1, the crosses of *D. cardinoides* and *D. polymorpha* are placed together as experiment No. 1, those of *D. neocardini* and *D. polymorpha* as experiment No. 2, and of *D. neocardini* and *D. cardinoides* as experiment No. 3. It is clear from the data that cross-insemination between the three species occurs very seldom or not at all, even when females and males of these species remain together for as long as a month. When

females and males of the same species are placed together, most females are inseminated within six days.

To discover whether interspecific matings ever take place, a few crosses were attempted using larger numbers of flies and more crowded conditions. In experiment No. 4 (Table 1), 100 virgin females of one species were placed together with 100 males of another species. The flies were transferred to fresh food every three days, and after approximately a month the still surviving females were examined for the presence of sperm in their seminal receptacles. Larvæ appeared in one of the bottles, but further tests proved these to be due to an accidental inclusion of a non-virgin female. (This experimental error is not included in the table.) One female, in the cross of *D. cardinoides* Belem females to *D. polymorpha* Araras males, proved to be inseminated, but no hybrid larvæ were produced by it.

In experiment No. 5 (Table 1), ten virgin females were placed with ten males of a different species in  $3\frac{3}{4} \times 1$ " vials with food. Altogether about fifteen vials of each cross were set up. Larvæ were produced in two vials, but these again were due to an accidental inclusion of non-virgin females, and are not included in the table. Two females in the cross of *D. cardinoides* Belem females to *D. polymorpha* Araras males were inseminated, as were also two females in the cross of *D. polymorpha* Bertioiga females to *D. cardinoides* Guatemala males. No viable hybrid larvæ appeared however in the vials containing these interspecifically inseminated females.

On the basis of our crosses, the strains from Belem, Guatemala, Iporanga, Mogi, and Teffe obviously belong to one species, those from Bertioiga and Araras to another, and the second strain from Teffe to a third.

The species so defined show very strong psychological or sexual isolation. Interspecific matings occasionally occur, but in no case are offspring produced.

Four strains of *D. cardinoides* were further tested in order to discover whether an incipient sexual isolation is present within the species. Since the strain from Iporanga differs slightly from the others in coloration (see below) it was thought that this form might be slightly isolated from the others. When males of one

TABLE 1  
 INTERSPECIFIC MATINGS WITHOUT CHOICE OF MATES

Experiment number	Species	Females	Males	Inseminated	Uninseminated	Males surviving
1.	cardinoides × polymorpha	Araras	Belem	0	14	13
		Belem	Araras	0	27	20
		Araras	Iporanga	0	31	20
		Iporanga	Araras	0	32	24
		Araras	Mogi	0	18	20
		Mogi	Araras	0	15	7
		Araras	Guatemala	0	11	7
		Guatemala	Araras	0	7	16
		Araras	Teffe	0	6	8
		Teffe	Araras	0	7	7
		Bertioga	Belem	0	12	3
		Belem	Bertioga	0	10	10
		Bertioga	Guatemala	0	19	27
		Guatemala	Bertioga	0	12	16
		Bertioga	Iporanga	0	10	26
Iporanga	Bertioga	0	39	23		
Bertioga	Mogi	0	8	4		
Mogi	Bertioga	0	14	10		
Bertioga	Teffe	0	6	3		
2.	neocardini × polymorpha	Araras	neocardini	0	39	27
		neocardini	Araras	0	11	34
		Bertioga	neocardini	0	13	19
		neocardini	Bertioga	0	22	27
3.	cardinoides × neocardini	neocardini	Belem	0	6	17
		Belem	neocardini	0	17	13
		neocardini	Guatemala	0	12	13
		Guatemala	neocardini	0	13	15
		neocardini	Iporanga	0	8	15
		Iporanga	neocardini	0	13	7
		neocardini	Mogi	0	15	15
Mogi	neocardini	0	16	18		
4.	cardinoides × polymorpha	Araras	Belem	0	75	50
		Belem	Araras	1	56	68
		Guatemala	Bertioga	0	54	50
		Bertioga	Guatemala	0	36	42
		Bertioga	Iporanga	0	39	41
Iporanga	Bertioga	0	43	38		
5.	cardinoides × polymorpha	Araras	Belem	0	110	154
		Belem	Araras	2	110	164
		Bertioga	Guatemala	2	70	84

strain of a species of *Drosophila* are given a choice of females of their own and of a foreign strain of the same species, preferential mating sometimes takes place (Dobzhansky and Streisinger 1944). The technique used in the present studies is very similar to that described by Dobzhansky and Streisinger (1944). Virgin females and males were collected. Since three of the strains are indistinguishable in appearance, the left wing of one class of females, the right wing of the other class was clipped. The flies, unlike in the experiments of Dobzhansky and Streisinger were

TABLE 2

NUMBER OF FEMALES DISSECTED (n) AND PER CENT CARRYING SPERM (PER CENT) IN VARIOUS CROSSES OF *D. cardinoides*

Females	Males	Homo- gamic		Hetero- gamic		$\chi^2$	Isola- tion index
		n	Per cent	n	Per cent		
Guatemala, Belem .....	Belem	30	33.3	42	19.0	1.9	0.27
Guatemala, Belem .....	Guatemala	29	55.1	29	62.0	0.3	-0.06
Guatemala, Iporanga...	Iporanga	57	73.7	57	8.7	49.6	0.78
Guatemala, Iporanga...	Guatemala	37	2.7	37	64.9	31.8	-0.92
Guatemala, Mogi .....	Mogi	50	92.0	46	39.1	30.2	0.40
Guatemala, Mogi .....	Guatemala	36	63.9	37	37.8	5.0	0.26
Belem, Iporanga .....	Belem	52	76.9	57	64.9	1.9	0.09
Belem, Iporanga .....	Iporanga	68	39.7	63	60.3	5.6	-0.20
Belem, Mogi .....	Belem	78	71.8	72	47.2	9.4	0.21
Belem, Mogi .....	Mogi	55	40.0	41	80.0	15.7	-0.33
Iporanga, Mogi .....	Iporanga	51	58.8	58	32.8	7.4	0.28
Iporanga, Mogi .....	Mogi	74	41.9	64	46.9	0.3	-0.06

aged separately for eight days, after which ten females and ten males of one kind, and ten females of the other kind, were placed into a vial with food. After six hours, by which time about half the total number of flies were usually fertilized, the females were dissected and examined for the presence of sperm.

The results of these experiments are summarized in Table 2. The Isolation Index, proposed by Stalker (1942), represents the differences of the percentages of homogamic and heterogamic females inseminated, divided by the sum of these percentages. The nearer the isolation index is to 1, the greater the preference for matings within the strain. Negative isolation indices show preference for heterogamic matings. The results reported in Table 2



show that representatives of different geographic strains of *D. cardinoides* do not necessarily interbreed at random. Thus, both Guatemala males and Iporanga males inseminate more Iporanga than Guatemala females. Mogi males inseminate more Mogi females than Guatemala females, but the reverse relation does not hold. Belem males, as well as Mogi males, inseminate more Belem

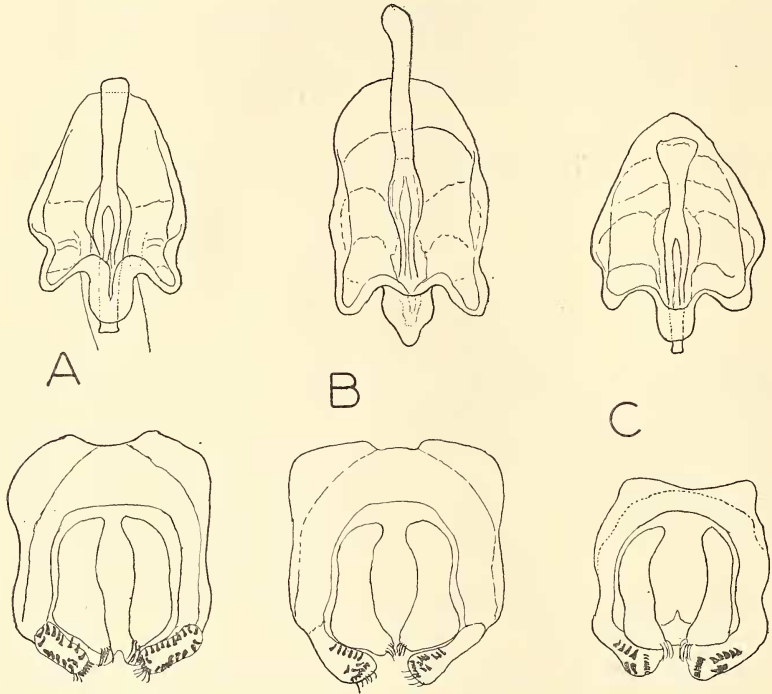


FIG. 1. Camera lucida drawings of the male genitalia of *Drosophila polymorpha* Dobzhansky and Pavan (A), *Drosophila cardinoides* Dobzhansky and Pavan (B), and *Drosophila neocardini* sp. nova (C). In each case, the penis apparatus is shown above and the genital arch and the anal plates below.

than Mogi females. These mating preferences do not seem to be correlated with the geographic origin of the strains. They are of course very much less pronounced than the preferences exhibited by interspecific matings in which only very few heterogamic inseminations occur. All intraspecific matings produce a multitude of larvæ which give rise to fertile adults. Interspecific inseminations seem to produce no larvæ at all.

The externally visible differences between the species are slight. Dobzhansky and Pavan (1943) indicate that *D. cardinoides* has a darker body color than *D. polymorpha*. This difference is indeed pronounced between the Iporanga strain of the former species (from which the type came) and the Bertioga and Araras strains of *D. polymorpha*. However, the Belem, Mogi, and Guatemala strains of *D. cardinoides* are only slightly darker in body color than the *D. polymorpha* strains, and distinctly lighter than the Iporanga strain of *D. cardinoides*. Thus the body color does not suffice to distinguish these species. *D. neocardini* is slightly lighter in body color than *D. polymorpha*. The black abdominal markings vary greatly from specimen to specimen within each strain of *D. polymorpha*, in *D. cardinoides* these markings are less variable and more or less resemble one of the color phases of *D. polymorpha*. *D. neocardini* has the abdominal black markings much reduced in extent compared to the lightest *D. polymorpha* and *D. cardinoides*.

The most striking differences, which can be used to distinguish the three species without breeding experiments, reside in the structure of the male genital organs, which are illustrated in Figure 1. These were obtained by clearing the whole flies in 10 per cent KOH, then dissecting out the genital organs under a low power microscope. The drawings were made from freshly dissected organs, with the aid of a camera lucida.

A description of *Drosophila neocardini*, made according to the form used in the modern *Drosophila* systematics, is given below.

***Drosophila neocardini*, species nova.**

♂, ♀. Arista with 8–10 branches. Antennæ yellow, third segment darker. Front velvety yellow, orbits and ocellar triangle rather shiny. Anterior and posterior orbitals equal, middle orbital  $\frac{1}{4}$  to  $\frac{1}{3}$  of other two, slender. Two prominent equally long oral bristles, occasionally three on one side. Face yellow. Carina large, not sulcate. Cheeks pale yellow, their greatest width about  $\frac{1}{2}$  diameter of the eyes. Eyes red with a short light pile.

Achrostical hairs in six rows. No prescutellars. Anterior scutellars convergent. Thorax shiny yellow without pattern. Pleuræ pale yellow. Anterior sternopleural  $\frac{1}{2}$  to  $\frac{2}{3}$ , middle sternopleural  $\frac{1}{4}$  to  $\frac{1}{3}$  of posterior. Legs yellow, apicals on first and second tibiæ, preapicals on all three.

Abdomen, yellow, shiny. Second, third, and fourth tergites with narrow black marginal bands interrupted in the middle, and occasionally expanded

on the sides toward the anterior margin which is seldom touched, the bands on the fourth tergite showing a tendency to break into four separate spots.

Wings clear or slightly brownish, crossveins not clouded. One or two prominent bristles at the apex of the first costal section, third costal section with bristles on its basal  $\frac{1}{3}$ . Costal index 3.6 (3.17-3.96), 4th vein index 1.6 (1.44-1.75), 5x index 1.5 (1.20-1.70).

♂. Length of body 2.2 mm. (2.0-2.4 mm.). Length of wing 2.2 mm. (2.0-2.3 mm.).

Testes with 7 outer and 5 inner coils, and a very long, slender vasa efferentia. Sperm pump with two long diverticula. Spermathecae weakly chitinized, yellow, resembling an inverted flower vase. Ventral receptacle with several dozen slender and thicker coils.

Eggs—four slender acuminate filaments.

Puparia—reddish brown. Horn slender and delicate, frequently glued to the surface of the pupa case. Horn index about six, anterior spiracle with many long and slender branches.

Chromosomes—metaphase plates show two pairs of V's, a pair of rods, and a pair of small dots.

Distribution—collected at Teffe (state of Amazonas), Brazil, by Prof. Th. Dobzhansky.

The type is deposited in the American Museum of Natural History.

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#### SUMMARY

Three species of the *cardini* group, namely *Drosophila polymorpha* Dobzhansky and Pavan, *D. cardinoides* Dobzhansky and Pavan, and *D. neocardini* sp. n., exhibit nearly complete sexual isolation from each other. When, very rarely, interspecific inseminations do occur, no viable offspring are produced. External morphological differences between these species are slight, but satisfactory differences in the structure of the male genitalia have been found.

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